AssayMax™

Rat Ceruloplasmin ELISA Kit

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For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Assay Summary

Step 1. Add 25 µl of Standard or Sample and 25 µl of Biotinylated Protein per well.
Incubate 2 hours.

Step 2. Wash, then add 50 µl of SP Conjugate per well.
Incubate 30 minutes.

Step 3. Wash, then add 50 µl of Chromogen Substrate per well.
Incubate 15 minutes.

Step 4. Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Symbol Key

Consult instructions for use.

Assay Template

	A	B	C	D	E	F	G	H
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Rat Ceruloplasmin ELISA Kit

Catalog No. ERC4001-1

Sample insert for reference use only

Introduction

Ceruloplasmin is an abundant alpha-2-serum glycoprotein that contains 95% of the copper found in the plasma of vertebrate species (1). Ceruloplasmin is a copper-binding protein that normally removes iron from cells by its ferroxidase activity. Ceruloplasmin concentration on average is 14.6 (± 4.0) mg/dl (2). Low levels of ceruloplasmin lead to the abnormal deposition of iron in cells, including those of the pancreas, liver, retina, and the basal ganglia region of the brain (1, 3-5).

Principle of the Assay

The AssayMax Rat Ceruloplasmin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of rat ceruloplasmin in **plasma and serum samples**. This assay employs a quantitative **competitive enzyme immunoassay** technique that measures rat ceruloplasmin in less than 3 hours. A polyclonal antibody specific for rat ceruloplasmin has been pre-coated onto a 96-well microplate with removable strips. Ceruloplasmin in standards and samples is competed by a biotinylated ceruloplasmin sandwiched by the immobilized antibody and streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This product is for **Research Use Only** and is Not For Use In Diagnostic Procedures.
- Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated protein, and SP conjugate) as instructed, prior to running the assay.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- Spin down the SP conjugate vial before opening and using contents.
- The Stop Solution is an acidic solution.
- The kit should not be used beyond the expiration date.

Reagents

- **Rat Ceruloplasmin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against rat ceruloplasmin.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Rat Ceruloplasmin Standard:** Rat Ceruloplasmin in a buffered protein base (25 μg, lyophilized).
- **Biotinylated Rat Ceruloplasmin:** 1 vial, lyophilized.
- **MIX Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 μl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard and Biotinylated Protein at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 μl, 20-200 μl, 200-1000 μl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation, and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:400 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes, and remove serum. Dilute samples 1:400 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Refer to Sample Dilution Guidelines below for further instruction.

Guidelines for Dilutions of 1:100 or Greater (*for reference only; please follow the insert for specific dilution suggested)*	
1:100	**1:10000**
A) 4 ul sample: 396 µl buffer(100x) = 100 fold dilution *Assuming the needed volume is less than or equal to 400 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) = 10000 fold dilution *Assuming the needed volume is less than or equal to 400 µl.*
1:1000	**1:100000**
A) 4 µl sample : 396 µl buffer (100x) B) 24 µl of A : 216 µl buffer (10x) = 1000 fold dilution *Assuming the needed volume is less than or equal to 240 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) C) 24 µl of B : 216 µl buffer (10x) = 100000 fold dilution *Assuming the needed volume is less than or equal to 240 µl.*

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 25 µg of Rat Ceruloplasmin Standard with 0.5 ml of MIX Diluent to generate a 50 µg/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (50 µg/ml) 1:2 with MIX Diluent to

produce 25, 12.5, 6.25, 3.125, 1.563, and 0.781 μg/ml solutions. MIX Diluent serves as the zero standard (0 μg/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[Rat Ceruloplasmin] (μg/ml)
P1	Standard (50 μg/ml)	50.00
P2	1 part P1 + 1 part MIX Diluent	25.00
P3	1 part P2 + 1 part MIX Diluent	12.50
P4	1 part P3 + 1 part MIX Diluent	6.250
P5	1 part P4 + 1 part MIX Diluent	3.125
P6	1 part P5 + 1 part MIX Diluent	1.563
P7	1 part P6 + 1 part MIX Diluent	0.781
P8	MIX Diluent	0.000

- **Biotinylated Rat Ceruloplasmin (1x):** Reconstitute Biotinylated Rat Ceruloplasmin with 4 ml MIX Diluent to produce a working solution. Allow the biotin to sit for 10 minutes with gentle agitation prior to use. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 25 μl of Rat Ceruloplasmin Standard or sample per well, and immediately add 25 μl of Biotinylated Rat Ceruloplasmin to each well (on top of the standard or sample) and tap plate to mix gently. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 μl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with

300 μl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.

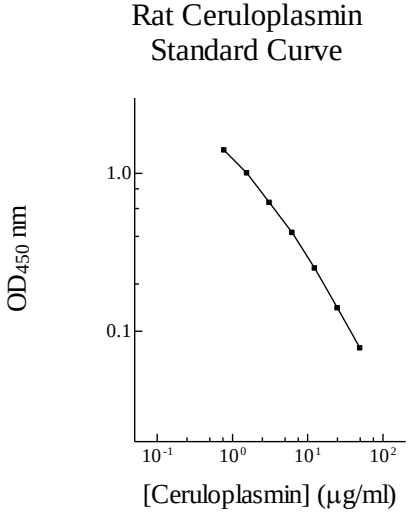
- Add 50 μl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μl of Chromogen Substrate per well and incubate for 15 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at low concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
  To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Precision, Sensitivity, and Specificity

- The minimum detectable dose of rat ceruloplasmin is typically ~ 0.7 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.4% and 7.0% respectively.

Linearity

Sample Dilution	Plasma	Serum
1:200	89%	90%
1:400	99%	98%
1:800	104%	103%

Recovery

Standard Added Value	1 – 20 µg/ml
Recovery %	83 – 111%
Average Recovery %	96%

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Bovine	None
Monkey	None
Mouse	<5%
Human	None
Swine	None

References

(1) Harris, Z. L, et al. *Proc. Natl. Acad. Sci.* Vol. 92, pp. 2539-2543, March 1995
(2) Aliyazicioğlu, Y et al. *The Turkish Journal of Pediatics* 2007; 49: 52-54
(3) Czaja, M. J et al. *J. Clin. Invest.* Volume 80, October 1987, 1200-1204
(4) Kumar, A et al. *WJM, October 1995-Vol 163, No. 4*
(5) Moller, L.B. et al. *Am. J. Hum. Genet. 66:1211–1220, 2000*

Version 1.3R3

Related Products

- EC4101-1 AssayMax Human Ceruloplasmin ELISA Kit (Urine, Saliva, Milk, and Cell Culture samples)
- EC4001-1 AssayMax Human Ceruloplasmin ELISA Kit (Plasma and Serum Samples)
- ERC4101-1 AssayMax Rat Ceruloplasmin ELISA Kit (Urine and Cell Culture samples)